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(54) Cleavable immunoconjugates for the delivery and release of agents in native form.

(57) The present invention provides a cleavable immunoconjugate whose linker contains a labile bond that is cleavable under a variety of mild conditions, including weakly acidic. Since the agent may be bonded directly to the linker, cleavage can result in release of native agent. The invention also provides methods for producing a cleavable immunoconjugate. Preferred agents include drugs, toxins, biological response modifiers, radiodiagnostic compounds, radiotherapeutic compounds, and derivatives thereof. The antibody employed in the invention may be an intact molecule, a fragment thereof, or a functional equivalent thereof. In a preferred embodiment, the specific antibody is a monoclonal antibody directed towards a tumor-associated antigen in man. The invention further provides a method for delivering to the cytoplasm of a target cell an agent free of its antibody carrier. A diagnostically or therapeutically effective dose of a cleavable immunoconjugate is administered to a mammal such as man.

Another aspect of the invention provides a method for isolating a compound containing an available nucleophilic group, such as a free sulfhydryl, amino, or hydroxyl group. The compound binds covalently to a solid phase which has been derivatized with the linker described above and is released in native form by a variety of mild conditions.

An additional aspect of the invention provides a method for introducing into a compound a free sulfhydryl, amin , or hydroxyl group by use of a reag nt structurally related to the linker describ d above. Preferred uses of the method are to add a free amino or a free sulfhydryl group to a protein, such as an antibody. This method has broad application in the field of compound modification, especially protein modification.

EP 0 318 948 A2

CLEAVABLE IMMUNOCONJUGATES FOR THE DELIVERY AND RELEASE OF AGENTS IN NATIVE FORM

Technical Field

The present invention relates generally to cleavable immunoconjugates that permit release of an agent in native form under mild conditions and to methods for making and using these conjugates.

Background of the Invention

A reoccurring problem in medicine in that, due to the lack of specificity of the agents used for treatment of illnesses, the patient is often the recipient of a new set of maladies from the therapy. This scenario is common especially in the treatment of the various forms of cancer.

An approach taken to circumvent the nonspecificity of the agents used to treat diseases is to couple an agent to a carrier that possesses some degree of specificity. A number of molecules have been utilized as carriers in agent delivery systems, but with limited success. Carrier molecules such as liposomes, proteins, and polyclonal antibodies have been used in conjunction with a broad spectrum of pharmaceutical or cytotoxic agents including radioactive compounds, agents which bind DNA, antimetabolites, agents which act on cell surfaces, and protein synthesis inhibitors.

With the discovery of a method to isolate antibodies with a single specificity, i.e., monoclonal antibodies (MAbs), came the hope that agents could now be delivered to selected cells via "immunoconjugates." "Immunoconjugates" are covalently bonded hybrid molecules composed of a recognition portion, such as an antibody molecule, an antibody fragment, or a functional equivalent thereof, and a biologically active portion, such as a toxin, toxin fragment, a drug, a biological response modifier, or a radioisotope. Immunoconjugates have enormous potential as potent anti-tumor agents, due to the selectivity imparted to the hybrid molecules by the antibody portion of the immunoconjugate. The exquisite selectivity of antibodies or antibody fragments permits delivery of increased doses of cytotoxic, inhibitory or radiolabeled moieties to a defined population of cells.

Although the MAb carrier systems have gone far to solve the cell-specificity problem, other problems remain. In particular, the design of the compound used to link the agent to the MAb is important. First, where the agent is only active, or at least more potent, when free from the MAb carrier, the linker needs to be cleavable in order to release the agent. Second, where the agent is only active, or at least more potent, when none of the linker remains attached following the cleavage, the labile bond must be the one formed between the linker and the agent. Third, the type of labile bond used should be chosen on the basis of the location, i.e., inside or outside a cell, of the release-inducing factor.

A number of different cleavable linker groups have been described previously. The mechanisms for release of an agent from these linker groups include cleavage by reduction of a disulfide bond, by irradiation of a photolabile bond, by hydrolysis of derivatized amino acid side chains, by serum complement-mediated hydrolysis, and acid-catalyzed hydrolysis. Some of these mechanisms are susceptible to release of the agent prior to having reached the specific cell, tissue or organ. Other of these mechanisms will provide faithful external delivery, however, they are inappropriate where the actual target site of the agent is inside a cell. Where an agent activates or inactivates a cell by binding to an intracellular component, the carrier-agent conjugate must be internalized and then the agent released.

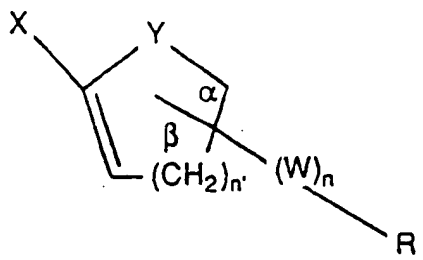
A way to achieve internalization of a carrier-agent conjugate is to take advantage of a cell's receptor-mediated endocytosis pathway. Antibodies are one example of a carrier that will bind to cell surface receptors and be internalized. Receptors which are internalized by receptor-mediated endocytosis pass through acidified compartments known as endosomes or receptosomes. Thus, the carrier-agent conjugate will be exposed transiently to an acidic pH.

Blattler et al., in U.S. Patent No. 4,569,789, describe a drug delivery system which is formed by reaction of an active substance with a maleic anhydride moiety. The active substance is released upon cleavage of the amide bond. The patent purports that cleavage occurs under mildly acidic conditions, yet the patent discloses that at pH 5 only about, 15% is cleaved after five hours. Even at pH 4 for five hours, less than 50% is cleaved.

Thus, there is a need in the art for a carrier-agent conjugate which releases the agent by cleavage under mild conditions. The present invention fulfills this need and further provides other related advantages.

Summary of the Invention

Briefly stated, the present invention, in one aspect, provides for a method of producing cleavable immunoconjugates comprising the steps of reacting an agent with a compound having the formula (I):

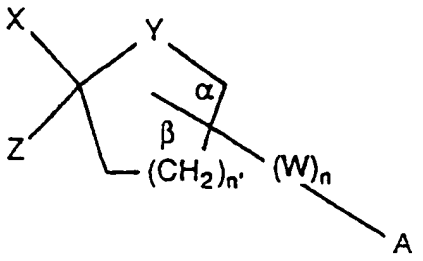


(I)

where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S, or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2; and
X is an H, alkyl group of C_6 or less, or alkoxy group of C_6 or less.

The agent adds to the carbon-carbon double bond, thereby forming a derivatized agent. The derivatized agent is conjugated with an antibody or antibody fragment for delivery to a cell, thereby forming the cleavable immunoconjugate. A variation on this method is to reverse the order of addition of an agent to the compound and of conjugation of an antibody.

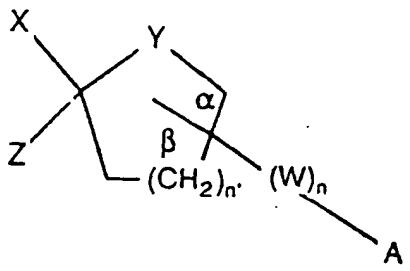
In another aspect, the invention provides a cleavable immunoconjugate. The immunoconjugate has the formula (II):



(II)

where: A is an antibody including the linking function, or antibody fragment including the linking function;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less; and
Z is an agent.

In yet another aspect, the present invention provides a method for delivering to the cytoplasm of a target cell an agent free of its antibody carrier. The method comprises the step of administering to a mammal a diagnostically or therapeutically effective dose of a cleavable immunoconjugate having the formula (II):

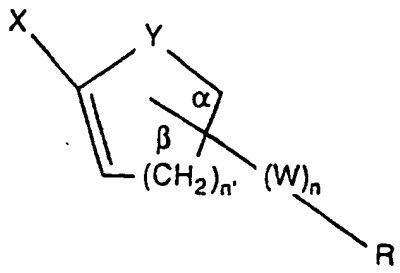


(II)

where: A is an antibody including the linking function, or an antibody fragment including the linking function;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less; and
Z is an agent.

Upon delivery of the immunoconjugate to a target cell, the bond joining the agent to the conjugate is cleaved, thereby releasing the agent. The bond is cleavable under a variety of conditions, including mildly acidic conditions or divalent cations, and is accelerated by heat. Since the agent may be bonded via one of its nucleophilic groups directly to the linker, cleavage can result in release of native agent.

A related aspect of the present invention provides a method for isolating a compound. The method comprises the steps for conjugating to a solid phase a reagent having the formula (I):

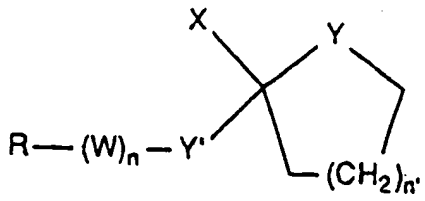


(I)

where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combinations thereof;
n is 0 to 30;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2; and
X is an H, alkyl group of C_6 or less, or alkoxy group of C_6 or less.

Thus, a derivatized solid phase is formed. The derivatized solid phase is contacted with a sample solution in which a compound containing an available nucleophilic group is present. The compound binds to the derivatized solid phase, thereby removing the compound from the sample solution. The bound compound is released from the derivatized solid phase by a variety of conditions, including mildly acidic conditions or divalent cations, and heat accelerates the reaction.

In yet another aspect, the present invention provides a method for introducing into a compound a free sulfhydryl, free amino, or free hydroxyl group. The method comprises the steps of reacting a compound with a reagent having the formula (III):



(III)

where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 30;
Y′ is S, O, or N;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less.
n′ is 1 to 2; and
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
to form a reagent-linked compound. The reagent-linked compound is cleaved at the bond between Y′ and the ring. Depending upon whether Y′ is an S, O, or N, a free sulfhydryl, free hydroxyl, or free amino group, respectively, will be added to the compound. The cleavage occurs under a variety of conditions, including mildly acidic conditions or divalent cations, and heat accelerates the reaction.

Other aspects of the invention will become evident upon reference to the following detailed description.

Detailed Description of the Invention

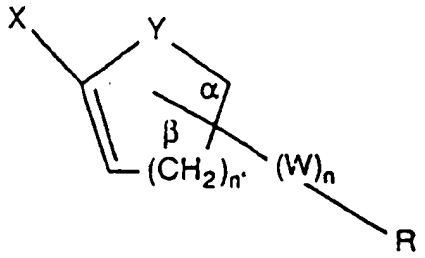
As noted above, the present invention provides cleavable immunoconjugates and a method for producing the immunoconjugates. Conjugates in which the carrier is an antibody, fragment thereof, or functional equivalent thereof, have enormous potential as potent anti-tumor agents. This is due to th selectivity imparted to the conjugate by the antibody portion. The exquisite selectivity of antibodies permits delivery of increased doses of agents, such as those that are cytotoxic, inhibitory, radiolabeled, or biological response modifiers.

One advantage of the immunoconjugates described is that cleavage occurs under mildly acidic conditions. For example, subjecting a conjugate to pH 5 results in substantially complete cleavage. The release mechanism may be described using as a model the example where as oxygen is the ring heteroatom. An equivalent of acid is thought to initially protonate the ring O. This is followed by formation of a stable carbocation. A second equivalent of acid results in the release of the agent and the formation of an aldehyde or ketone on the compound linking the agent to the antibody.

Another advantage of the immunoconjugates of the present invention is that cleavage results in the release of the agent without any of the linker remaining attached. Popular cleavable linkers are those bifunctional reagents with a disulfide bond interposed between two reactive end groups. Cleavage of the disulfide bond by the addition of a reducing compound leaves one end of the bifunctional reagent still attached to the agent. Many agents, however, are inactivated by the permanent addition of a linker or fragment thereof to their structures. The immunoconjugates of the present invention are cleaved at the bond formed between the agent and the compound linking the agent to the antibody. Therefore, the pr sent invention provides a way of releasing an agent in native form at the target site.

Yet another advantage of the present invention is the ease of preparation of the linking compound, du in part to the commercial availability of the reagents needed.

The present invention also provides a method for producing a cleavable immunoconjugate comprising the steps of reacting an agent, capable of addition to a carbon-carbon double bond, with a compound having the formula (I):



(I)

to form a derivatized agent and conjugating the derivatized agent with an antibody or fragment thereof to form the cleavable immunoconjugate.

The elements of th compound depicted above in formula I include the following. R is a chemically reactive moiety. The moi ty may be strongly electrophilic or nucleophilic and thereby be available for reacting directly with an antibody or fragment ther of. Alternatively, the moiety may be a weaker electrophile or nucleophile and therefore r quire activation prior to the conjugation with an antibody or

fragment thereof. This alternative would be desirable where it is necessary to delay activation of R until an agent is added to the compound in order to prevent the reaction of the agent with R. In either scenario R is chemically reactive, the scenarios differ by whether following addition of an agent, R is reacted directly with an antibody or fragment thereof or is reacted first with one or more chemicals to render R capable of reacting with an antibody or fragment thereof. A discussion of reactions illustrative of the activation of R is found below.

W in formula I is a group that functions as a "spacer arm" and may be useful to distance the antibody or fragment thereof from the agent. Groups which may be used include methylene (-CH_2-), methyleneoxy (-CH_2-O-), methylenecarbonyl (-CH_2-CO-), amino acids, or combinations thereof. The number, n, of groups such as these would be typically 0 to 30 and preferably 0 to 10. W, or R where n is 0, may be attached to one or the other of the ring positions designated as α and β. Because the number of methylene ring carbons at the β position is defined by n′ which may be greater than one, the β position includes additional points for attachment of a W or an R to the ring.

Y in formula I is a heteroatom. Preferred heteroatoms include oxygen (O), sulfur (S), or nitrogen (N). When nitrogen is the heteroatom, it should be in the form of a tertiary amine, i.e., NR′, such as where R′ is an alkyl group of C_6 or less. A particularly preferred heteroatom is O. The ring size of the compound may be increased above 5 by an increase in the number, n′, of ring methylene groups. Preferred ar 5-membered rings, such as a dihydrofuran derivative, and 6-membered rings. X may be a hydrogen (H) or another substituent, preferably an alkyl group of C_6 or less and an alkoxy group of C_6 or less.

The step of reacting an agent and a compound of formula I results in the attachment of the agent to the compound via addition to the carbon-carbon double bond on the ring. In this manner, a derivatized agent is formed. Any agent containing a group capable of reacting with the compound may be employed in the present invention. Preferred agents include drugs, toxins, biological response modifiers, radiodiagnostic compounds and radiotherapeutic compounds. An agent may be reacted in its native form or a derivative thereof. An example of a derivative form is where an amino group on an agent is modified by reaction with a compound, such as iminothiolane, to introduce a sulfhydryl group on the agent. Preferred toxins include ricin, abrin, diptheria toxin and Pseudomonas exotoxin A. Preferred radionuclides for the radiodiagnostic and radiotherapeutic compounds include ^{99m}Tc, $^{186/188}Re$, and $^{123/131}I$.

The step of conjugating may be performed by joining an antibody or fragment thereof to the derivatized agent by direct reaction with R. Alternatively, it may be desirable to include before the step of conjugation a preparatory step. For example, an antibody or fragment thereof may be itself derivatized in preparation for direct reaction with R. The derivatization of an antibody or antibody fragment includes reaction with any of the numerous bifunctional reagents reported in the literature.

A direct reaction with R by derivatized or underivatized antibody or fragment thereof is intended to mean that R is capable of reacting with the derivatized or underivatized antibody or fragment. For exampl , R may be a carbonyl-containing group, such as an anhydride or an acid halide, or an alkyl group containing a good leaving group, e.g., a halide. The latter class of compounds may be represented by alkyl X′, where X′ stands for the leaving group. As another example, R may be a nucleophilic group, such as an amino or sulfhydryl group, which is capable of reacting with a derivatized antibody or fragment, e.g., containing a maleimide group.

Yet another way to perform a step in preparation for conjugation of the derivatized agent with underivatized or derivatized antibody or antibody fragment is to convert R. Examples of conversions of R include where R is a carboxyl group and is then activated. Activation of a carboxyl group includes formation of an "active ester," such as a succinimidyl ester. The term "active ester" is known to refer to esters which are highly reactive in nucleophilic substitution reactions. In the present invention, the antibody or antibody fragment would be the nucleophile.

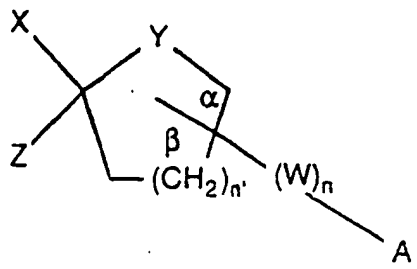
Another example of a conversion is where R is a succinimide derivative containing a protective group, such as phenylsulfonyl. Upon removal of the group, the succinimide is converted to a maleimide which is highly reactive in nucleophilic addition reactions. Alternatively, R may be an amino, sulfhydryl, or hydroxyl group and the conversion comprises reaction with a bifunctional reagent. It will be evident to one skilled in the art that a variety of bifunctional reagents, both homobifunctional and heterobifunctional, may be employed within the present invention.

The antibody employed in the present invention may be an intact molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab′)$_2$, Fab′, Fab. and Fv. Whil polyclonal antibodies may be employed in the present invention, monoclonal antibodies (MAbs) are preferred, especially those directed toward a tumor-associated antigen in man. Particularly preferred MAbs are anti-TAC, or other interleukin 2 receptor antibodi s; 9.2.27 and NR-ML-05 to human melanoma associated proteoglycan; NR-Lu-10 to 37-40 kilodalton pancarcinoma glycoprotein and OVB_3 to an as yet

unidentified ant[redacted] Genetically engineered antibodies or fragm ereof of these and other MAbs may be employed as well.

A variation on this method is to reverse the order of the addition of an agent and an antibody in the formation of an immunoconjugate. Specifically, first an antibody is conjugated to a compound, whose general structure is depicted above, via R and then an agent is reacted via addition to the double bond on the compound attached to the antibody. The above discussion regarding the compound, agent, antibody, and chemical reactions is relevant to this variation as well.

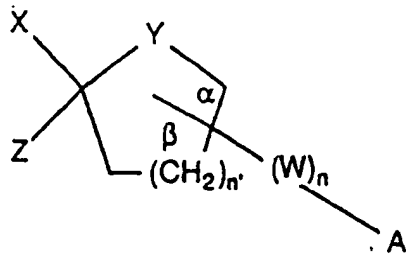
In addition to the methods provided, the present invention provides cleavable immunoconjugates having the formula (II):



(II)

where: W, n, Y, n', α, β, and X are defined as described above. A is an antibody or an antibody fragment, and for either it includes any linking function used to attach the antibody or fragment to yield the immunoconjugate. Preferred MAbs are described above. Z is an agent. Any agent capable of being covalently attached to the cleavable immunoconjugate may be employed in the present invention. Typically, an agent will be bonded to the immunoconjugate by use of a sulfhydryl, amino, or hydroxyl group on the agent. The agent may be bonded directly to the immunoconjugate or indirectly with a linking function interposed between the agent and the ring. Preferred agents include those described above.

An additional aspect of the present invention provides a method for delivering to the cytoplasm of a target cell an agent free of its antibody carrier. The method comprises the step of administering to a mammal a diagnostically or therapeutically effective dose of a cleavable immunoconjugate having the formula (II):



(II)

where W, n, Y, n', α, β, X, A, and Z are defined above. A preferred mammal is man. Preferred MAbs and agents include those described above.

The agent may be diagnostically and/or therapeutically effective. A preferred diagnostic agent is a compound containing ^{99m}Tc. A diagnostically effective dose of a cleavable immunoconjugate incorporating such an agent is generally from about 10 to about 30, typically from about 15 to about 25, and preferably from about 18 to about 20 mCi per 75 kg body weight. A preferred therapeutic agent is a toxin, such as Pseudomonas exotoxin A. A therapeutically effective dose of a cleavable immunoconjugate incorporating such as agent is generally from about 1 to about 100 and preferably from about 1 to about 10 ng per 75 kg body weight. The precise dose for a particular cleavable immunoconjugate is dependent upon the antibody used, as antibodies vary with respect to the number of receptors and their affinity for the receptors, and the agent used, as toxins, for example, vary with respect to their potency. It will be evident to one skilled in th art how to determin the optimal eff ctive dose for a particular cleavable immunoconjugate.

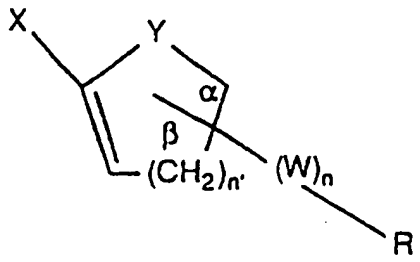
Th step of administering to a mammal a diagnostically or therapeutically effectiv dose of a cleavable immunoconjugate having formula II sets in motion a sequ nce of events in vivo that results in the agent portion of th immunoconjugate being d livered free of the antibody portion to the cytoplasm of a target

cell. The antibody or antibody fragment portion of the immunoconjugate imparts the selectivity which permits delivery to and binding at the surface of a specific cell. An immunoconjugate of formula II is susceptible to cleavage by pH less than or equal to 6.0 and the acid-catalyzed cleavage is accelerated by heating above room temperature, about 23°. Since antibodies bind to cell surface receptors which are internalized into the cytoplasm via acidified compartments, it is believed that the release to the cytoplasm of an agent from an immunoconjugate of the type described herein is the result of this transient exposure to acidic pH. Futher, because mammals such as man have normal body temperatures above 23° C, body heat may be a factor in the release. The delivery of an agent free of its antibody carrier to the cytoplasm of a targeted cell increases its potency as compared to the agent when irreversibly linked to its carrier. This method is useful to diagnose, stage, evaluate or treat diseases such as cancer in humans.

A related aspect of the present invention provides a method for isolating compounds containing an available nucleophilic group, such as a free sulfhydryl, free amino, or free hydroxyl group. The isolation of a compound, e.g., from a reaction mixture, is often a difficult and/or tedious process. It was widely beli ved that the attachment to a solid phase of a reagent with an affinity for a compound was the panacea for the problems with earlier isolation procedures.

In theory, the undesired compounds are removed simply by washing the solid phase. In practice, however, washing conditions sufficient to remove the impurities often result in removal of the compound of interest. This is due to the fact that the compound is only held to the solid phase by noncovalent interactions with the reagent. While covalent attachment of the compound to the solid phase is preferably from a washing standpoint, it can make recovery of the compound off the solid phase impossible. The method of the present invention provides a way to attach covalently a compound of interest and thereby facilitate removal of undesired compounds, yet nevertheless permit easy recovery of the compound of interest from the solid phase.

This method of isolating compounds containing an available nucleophilic group comprises the following steps. To a solid phase is conjugated a reagent having the formula (I):



(I)

to form a derivatized solid phase. The derivatized solid phase is contacted with a sample solution in which a compound containing an available nucleophilic group is present, such that the compound binds to the derivatized solid phase, thereby removing the compound from the sample solution. The compound bound to the derivatized solid phase may be released.

The elements of the reagent depicted above in formula I include the following: W, n, Y, n', α, β, and X, which are defined above. R is a chemically reactive moiety which may be a nucleophile or an electrophile. When the solid phase contains available nucleophilic groups, such as a free amino group, R is an electrophile such as an activated ester. Conversely, when the solid phase contains electrophilic groups, R is a nucleophile. Examples of suitable solid phases include controlled pore glass and preformed polymers, such as polyvinyls, polyacrylamides, polydextrans, and polystyrenes.

The step of conjugating the reagent to the solid phase attaches the former to the latter via R, thereby forming a derivatized solid phase. A sample solution, in which a compound containing an available nucleophilic group is present, is contacted with the derivatized solid phase. Examples of available nucleophilic groups include free sulfhydryl, free amino, and free hydroxyl groups. The step of contacting results in covalent attachement of the compound to the derivatized solid phase via addition to the carbon-carbon double bond on the reagent's ring. Following the step of contacting, it may be desirable to wash the solid phase to remove noncovalently bound compounds.

The covalently bound compound may b released in native form from the solid phase by a variety of ways. For example, cleavage of the bond formed between the compound and a ring-carbon of the derivatized solid phase may be achieved by mildly acidic conditions or divalent cations, and be accelerat d by heat. In particular, cleavage occurs by decreasing the pH of a solution contacting the solid phase to 6.0 or lower, by adding divalent cations such as Zn^{2+} at a concentration at least equimolar to that of the reagent

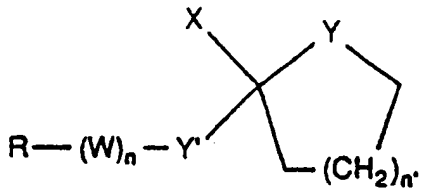
attached to th[redacted] phase, or by raising the temperature above [redacted] in the presence of pH 6.0 or lower.

Alternative[redacted] undesired compound or compounds, rather [redacted] a compound of interest, may contain an available nucleophilic group. In this situation, the undesired compound or compounds will bind to a derivatized solid phase and the compound of interest will not bind. Therefore, the method may be used to purify by a single technique one or more compounds from a reaction mixture so long as the compounds of interest or the impurities, but not both, contain available nucleophilic groups.

Another way to use this type of reagent to isolate a compound is to bind the compound to the reagent before contacting with a solid phase. For example, a sample solution in which a compound containing an available nucleophilic group, such as a free sulfhydryl, free amino, or free hydroxyl group, is present, may be reacted with a reagent whose general structure is as described above. The step of reacting results in covalent attachment of the reagent to the compound via addition to the carbon-carbon double bond on the reagent's ring. A reaction mixture is thereby formed, wherein a derivatized compound is present. The reaction mixture is then contacted with a solid phase capable of selectively binding, covalently or noncovalently, the derivatized compound, thereby removing the derivatized compound from the reaction mixture. The bound compound may be released in native form by a variety of ways, including those described above.

Yet another aspect of the present invention includes a method for introducing into a compound a fre sulfhydryl, free amino, or free hydroxyl group. The two reagents widely used for the introduction of -SH groups in proteins are S-acetyl mercaptoacetic acid succinimidate ester (SATA) and mercaptoacetyl succinic anhydride (SAMSA). In both cases, the antibody reacts to form an amide bond. In the case of SATA, the antibody after the reaction is stored as Ab-Lys-NH-$COCH_2SCOCH_3$ at -20° C from which free sulfhydryl (-SH) groups are generated by treatment with hydroxylamine. In the case of SAMSA, the amine from the protein reacts with the anhydride to form Ab-Lys-NHCO-CH_2-CH(SH)-COOH. Both reagents suffer from several disadvantages. First, the S-acetyl protecting group is base labile, i.e., can be hydrolyzed at pH 7-8. Ideally that is the pH necessary for the conjugation of antibody with electrophiles. Hydrolysis of thioacetyl groups effectively competes with displacement reactions. Second, thioesters are by thems lves active esters. Amines will react with thioesters to give N-acetyl compounds. Third, in the case of mercaptoacetyl succinic anhydride, reaction of Fab gives several components including aggregates. This results directly from competing reactions of the proteins with the reagents. The method of the present invention overcomes the above problems and, furthermore, reagents like hydroxylamine need not be used to generate the free -SH group.

This method for introducing into a compound a free -SH, -NH_2, or -OH group comprises the steps of reacting a compound with a reagent having the formula (III):



(III)

to form a reagent-linked compound and cleaving the reagent-linked compound at the bond between Y′ and the ring, thereby introducing into the compound a free sulfhydryl, free amino, or free hydroxyl group, depending upon whether Y′ is S, N, or O respectively.

The elements of the reagent depicted above in formula III include the following: W, n, Y, n′, and X, which are defined above. Y′ is a heteroatom. Preferred heteroatoms include sulfur (S), oxygen (O), or nitrogen (N). R is a chemically reactive moiety. The moiety may be a nucleophile or an electrophile. The selection is generally determined by whether the reactive group on a compound is nucleophilic or electrophilic. For example, where the reactive group on a compound is a nucleophile, such as an amino group, R is an electrophile such as an activated ester or an anhydride. An exception to this method of selecting R is where it is desired to form a disulfide bond between a compound and R. In that situation, a sulfhydryl group on the compound and a sulfhydryl group on the reagent, i.e., R is -SH, may be oxidized to form a disulfide bond. Because disulfide linkages are cleavable, e.g., by reducing agents, it is possible to create a reversibly modified compound. For example, where Y′ is N and R is -SH and the reagent and a compound are joined by a disulfide bond, the result is the addition to a compound of an amino group that can be remov d.

Any compound containing a functional group capable of reacting with the reagent via R may be

employed in the present invention. The functional group may be present on a native form of a compound or be added to it. Preferred compounds are proteins generally, e.g., antibodies.

When the compound is reacted with the reagent, the former is attached to the latter via R, thereby forming a reagent-linked compound. The reagent-linked compound is then cleaved at the bond between Y′ and a ring carbon, thereby forming compounds with free sulfhydryl, free amino, or free hydroxyl groups, depending upon whether Y′ is an S, N, or O, respectively. The step of cleaving may be achieved by a variety of ways, including exposing the reagent-linked compound to mildly acidic conditions, heat, or divalent cations. In particular, cleavage occurs by decreasing the pH to 6.0 or lower, by adding divalent cations such as Zn^{2+} at a concentration at least equimolar with the reagent-linked compound, or by raising the temperature to at least 37° C in the presence of pH 6.0 or lower. The preferred pH range is 5.0 - 6.0.

The step of cleaving results in Y′ remaining attached to the compound. Therefore, introduction of a desired free heteroatom into a compound is achieved by selection of the appropriate heteratom for Y′. For example, if the addition of a free amino group to a compound is desired, then the heteroatom selected for Y′ is N.

A preferred use of the method with proteins is to introduce a free sulfhydryl group. For example, an amino group of protein, e.g., a lysine residue, may be used as the nucleophilic group to react with a form of the reagent where Y′ is S. When the resulting reagent-linked protein is cleaved, the net effect is to convert a free amino group on the protein to a free sulfhydryl group. Another preferred use is to introduce an amino group.

To summarize the examples which follow, Example I provides the preparation of cleavable immunoconjugates utilizing 6-mercaptopurine and MAb 9.2.27. Example II describes testing for the in vitro cytotoxicity and the biodistribution and toxicology of the cleavable immunoconjugates utilizing Pseudomonas exotoxin. Example III discloses isolation of phenylmercaptoacetamide utilizing a derivatized agarose. Example IV describes the introduction of a free sulfydryl group into intact MAb 9.2.27.

The following examples are offered by way of illustration and not by way of limitation.

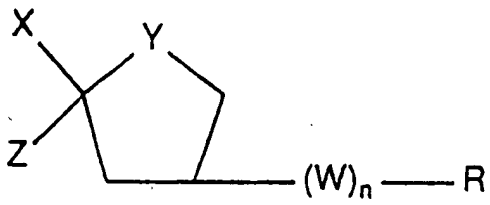
EXAMPLES

EXAMPLE I

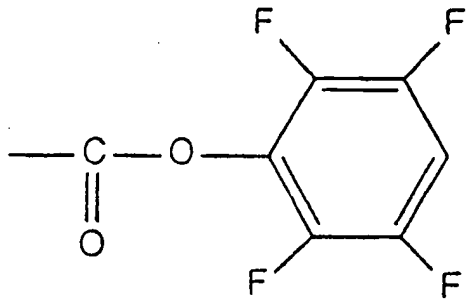
Preparation of Cleavable Immunoconjugates

A. Preparation of a Derivatized Agent

The derivatized agent having the following formula is prepared as described below:



where:

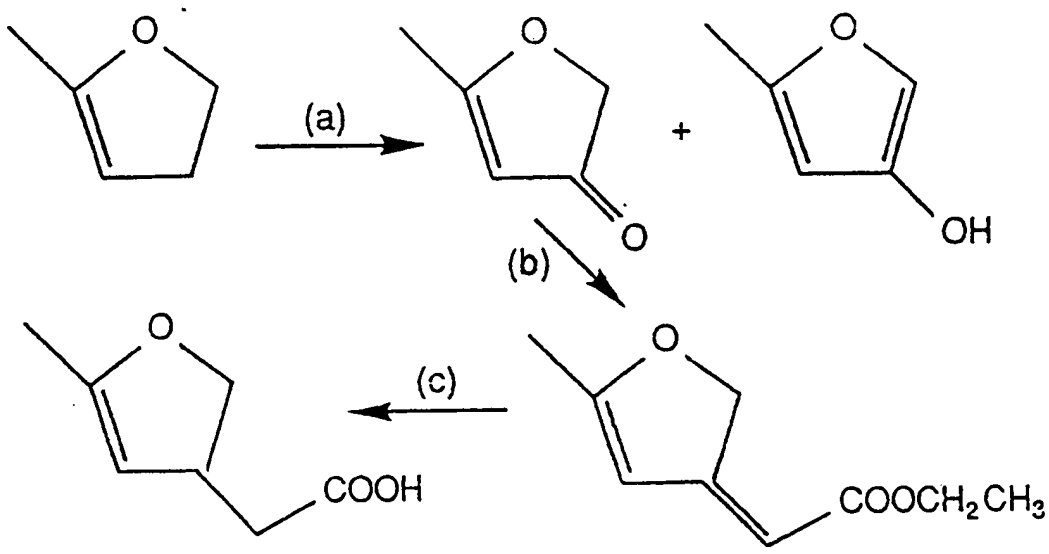
R = 

W = CH_2 -
n = 1
Y = oxygen
X = CH_3
Z = 6-mercaptopurine

1. Preparation of 2,3-dihydro-5-methyl-furan-3-yl acetic acid 1.

Compound 1 where n' = 1 is synthesized according to the procedure described by D.V. Banthorpe et al. (Phytochemistry 18:666-667, 1979) as depicted in Scheme I.

Scheme I

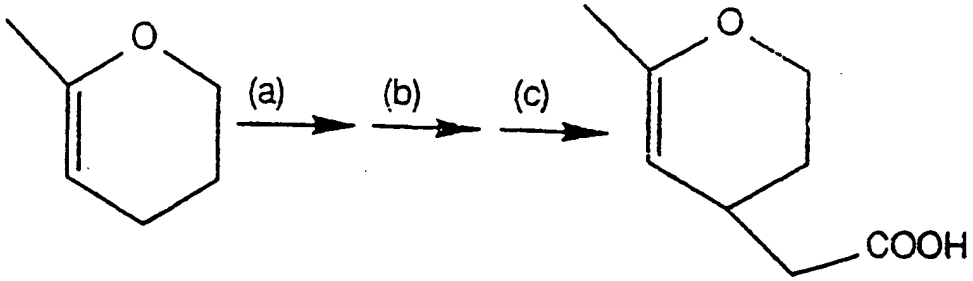


(a) SeO_2, dioxane

(b) $CH_3CH_2OOCH{=}PO_3$

(c) Raney nickel

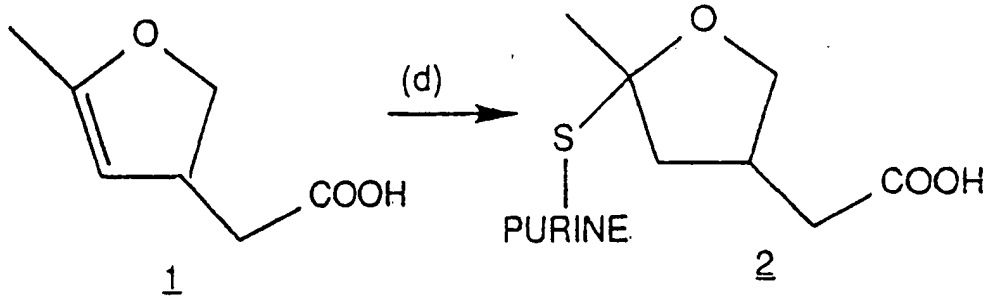
Similarly, the dihydropyran derivative 1B where $n' = 2$ is prepared using the analogous method described in Scheme I.



2. Reaction of An Agent with Compound 1.

A hemithioketal derivative, 2, of 6-mercaptopurine, an anti-tumor drug, where $n' = 1$ is prepared according to the pathway depicted in Scheme II below. Specifically, a mixture of 2,3-dihydro-5-methyl-furan-3-yl acetic acid 1 (1.0 equivalent) and 6-mercaptopurine (1.0 equivalent) in THF is treated with a catalytic amount of para-toluene sulfonic acid (0.01 equivalent). The resultant mixture is allowed to stir at room temperature overnight. The organic phase is washed with H_2O and dried over anhydrous $MgSO_4$. After removal of the solvent, a hemithioketal derivative of 6-mercaptopurine, product 2, is obtained.

Scheme II

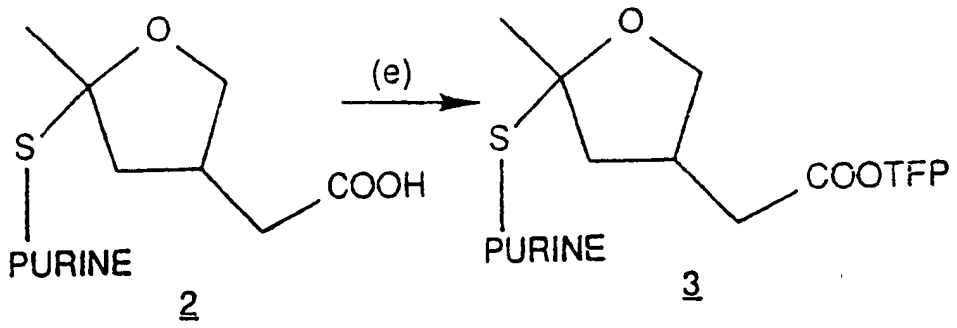


(d) 6-mercaptopurine, pTsOH, THF

3. Conversion of 2 to the tetrafluorophenyl ester 3.

An activated ester form, 3, of 2 is prepared according to the pathway depicted in Scheme III below. Specifically, the hemithioketal derivative of 6-mercaptopurine, 2, (1.0 equivalent) is combined with 2,3,5,6 tetrafluorophenol (TFP) (1.1 equivalent) in anhydrous THF. After addition of crystalline dicyclohexyl carbodiimide (DCC) (1.1 equivalent), the mixture is stirred for 12 hours at 25°C. After removal of the precipitated dicyclohexyl urea by filtration and removal of the THF under reduced pressure, the product is obtained. After chromatography on silica gel, product 3 is obtained.

Scheme III



(e) 2,3,5,6-tetrafluorophenol, DCC, THF

B. Conjugation of TFP ester 3 with Monoclonal Antibody 9.2.27

The monoclonal antibody 9.2.27 is directed to a melanoma-associated antigen and is prepared to

according to [redacted]rgan et al., Hybridoma 1:27-35, 1981. A meth[redacted]ic solution of 3 (25 µl total volume) is transferred to a vial containing buffered antibody solution (pH 8.5-10) of at least 1 mg antibody per 1 ml. The conjugation mixture is warmed at 37° C for 20 minutes. The modified antibody is purified either using gel permeation chromatography or small pore filtration (e.g., Centricon ultra centrifugation).

EXAMPLE II

A. In Vitro Cytotoxicity of Cleavable Immunoconjugates

Cleavable immunoconjugates, where Pseudomonas exotoxin (PE) is the agent, are prepared in accordance with a modification of the procedures of Example I.

Prior to reaction with compound 1 (Example I.A.2.), the carboxyl groups on PE are protected, e.g., by suspension of PE in O.1N methanolic HCl at room temperature overnight. PE is then treated with a reducing agent to provide a free sulfydryl for reaction with compound 1. A mixture of compound 1 (1.0 equivalent) and PE (1.0 equivalent) in an aqueous solution containing 10% dioxane is treated with a catalytic amount of para-toluene sulfonic acid (0.01 equivalent). The resultant mixture is allowed to stir at room temperature overnight and the derivatized PE is isolated by gel filtration chromatography. The succinimidyl ester of derivatized PE is prepared using N-hydroxysuccinimide and a water soluble carbodiimide. After separation by gel filtration chromatography, the carboxyl groups on PE are deblocked, e.g., by hydroxylamine, and PE is conjugated to Mab 9.2.27 according to Example I.B.

ADP-ribosylation is measured in a cell-free system according to the method of B.G. Vanness et al., J. Biol. Chem. 255:10717 (1980). The ADP-ribosylation activity of the cleavable PE immunoconjugates in the presence and absence of dilute acid or divalent cations is compared to the activities under the same conditions of PE alone and of non-cleavable PE immunoconjugates.

In vitro cytotoxicity testing is performed according to the method of A.C. Morgan, Jr. et al., JNCI 78:1101, 1987, using ^{3}H-leucine incorporation to measure protein synthesis inhibition due to ADP-ribosylation activity by PE. For testing of PE:9.2.27 conjugates, two human melanoma cell lines are utilized as targets - A375 met mix (antigen-positive) and A375 I^{0} Primary (antigen-negative). For assay of PE:anti-TAC conjugates, target cells are HUT 102 (antigen-positive) and CEM (antigen-negative) as discussed in D.J.P. Fitzgerald et al., J. Clin. Invest. 74:966 (1984). Conjugates are examined in two formats: (a) short exposure, wherein the conjugate is incubated with target cells for one hour at 37° C, the monolayer gently washed, and the cultures continued for up to 72 hours before the addition of ^{3}H-leucine; and (b) long exposure, wherein the conjugate is added and the target cells exposed for the entire length of the culture period.

B. Biodistribution and Toxicology of Cleavable Immunoconjugates

Tumor localization and biodistribution of conjugates are examined in a nude mouse xenograft model of human melanoma, according to the method of K.M. Hwang et al., Canc. Res. 450:4150, 1985. PE is radiolabeled with ^{125}I-para-iodophenyl (PIP) as shown by D.S. Wilbur et al., J. Nucl. Med. 27:959 (1986). This radiolabel is not subject to dehalogenation, and thereby can be used to more effectively follow the biodistribution of conjugates. The radiolabeled PE is conjugated to a monoclonal antibody, such as 9.2.27 or anti-TAC, using the linkers of the present invention as described in Example I. Animals are sacrificed at 20 hours post-injection, and organs are blotted, weighed and counted. A %dose per gram is calculated for each tissue. In addition, serum half-life is estimated by retroorbital sampling of whole blood.

Mice are administered different doses of PE-anti-TAC and PE:9.2.27 conjugates intraperitoneally to determine an LD_{100}. Following administration of radiolabeled PE immunoconjugates, the resultant tumor localization and biodistribution of the conjugates are determined. Non-specific toxicity of PE:anti-TAC conjugates is also assessed in cynomolgous monkeys. Monkeys are monitored for liver enzyme levels, and are obs rved for other relevant symptoms, including appetite, presence/absence of nausea, and temperature.

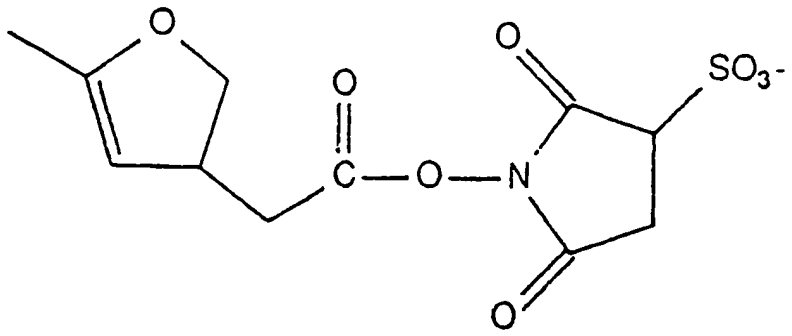
EXAMPLE III

Isolation of a Compound Containing an Available Nucleophilic Group By a Derivatized Solid Phase

A. Preparation of a Derivatized Solid Phase

1. Synthesis of Reagent, 4, for Derivatization of Solid Phase.

Compound 1, 2,3-dihydro-5-methyl-furan-3-yl acetic acid, is synthesized as described in Example I. To a solution of 1 (1.0 equivalent) in dry dimethylformamide (DMF) is added crystalline dicyclohexyl carbodiimide (DCC) (1.1 equivalent), followed by the addition of N-hydroxysulfosuccinimidate (1.1 equival nt). After stirring at room temperature for 12 hours, the precipitated dicyclohexylurea (DCU) is removed by gravity filtration. The DMF is removed under reduced pressure and the resultant residue is dissolved in a minimal amount of 50:50 acetonitrite-H_2O. This compound 4 is then purified using silica gel chromatography.



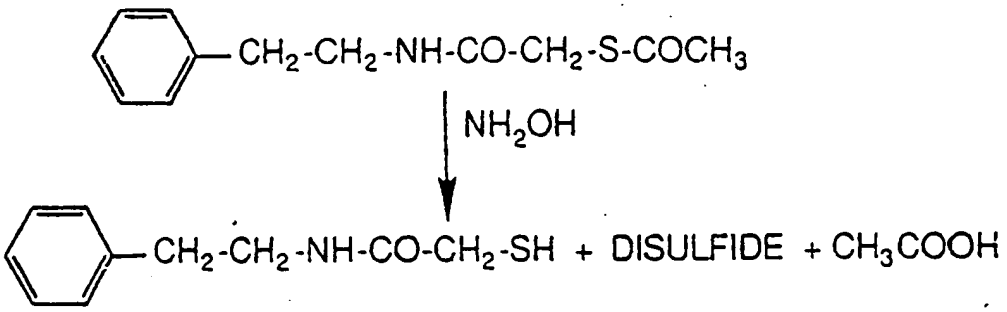
4

2. Coupling of 4 with AH-Sepharose-4B

Aminohexyl (AH)-Sepharose-4B is obtained from Pharmacia and swelling and washing of the gel is carried out according to the procedure in Affinity Chromatography -Principles and Methods, Pharmacia Publication, June 1979, pp. 22-23.

The above sulfosuccinimidate 4 is dissolved in bicarbonate buffer (pH = 8) containing 5-10% acetonitrite at a concentration is excess of the spacer groups. The solution is added to the gel and pH is readjusted if necessary during the period of coupling, which is done overnight. The Sepharose-spacer-4 conjugate is stored at 4° C.

B. Isolation of Phenethylmercaptoacetamide With the Derivatized Solid Phase



A solution of S-acetylphenethylthioacetamide is treated with excess hydroxylamine in methanol:water (1:1) to yield a mixture of products and unreacted starting material, as depicted above. The solution is passed through a column containing the derivatized Sepharose prepared in part A above. The thiol in the mixture (phenethylmercaptoacetamide) is retained in the column by formation of a covalent adduct, while the other compounds in the mixture (excess hydroxyl amine, the disulfide and unreacted S-acetylphenethyl-thioacetamide) are eluted.

The desired thiol is freed from the column by passing a pH 4-5 buffer through the column.

EXAMPLE IV

Introduction of a Free Sulfhydryl Group into an Antibody

A. Preparation of Hemithioketal Derivative of Monoclonal Antibody 9.2.27

1. Synthesis of a Hemithioketal.

A mixture of 2-methyl-4,5-dihydrofuran and 2-mercaptoacetic acid in THF are treated with a catalytic amount of para-toluene sulfonic acid according to the procedure in Example I.A.2. After removal of the solvent, S-(2-methyl-tetrahydrofuran-2-yl) mercaptoacetic acid is obtained. Alternatively, mercaptosuccinic acid is reacted with 2-methyl-4,5-dihydrofuran and then converted to a mercaptosuccinic anhydride derivative by a dehydrating agent such as dicyclohexyl carbodiimide.

The derivatized mercaptoacetic acid is converted to the tetrafluorophenyl (TFP) ester as described in Example I.A.3. Alternative active esters are formed by reaction with dicyclohexyl carbodiimide and N-hydroxysuccinimide or N-hydroxysulfosuccinimidate.

2. Conjugation of TFP ester to Monoclonal Antibody 9.2.27

Monoclonal antibody 9.2.27 is prepared according to the method described in Example I.B. A methanolic solution of an activated ester form of the mercaptoacetic acid derivative (25 µl total) is transferred to a vial containing a pH 8.0 buffered antibody solution. The antibody concentration is at least 1.0 mg/ml. The reaction mixture is incubated at 37° C for 20 minutes. The derivatized antibody is purified using gel permeation chromatography or small pore filtration.

B. Generation of Free Sulfhydryls

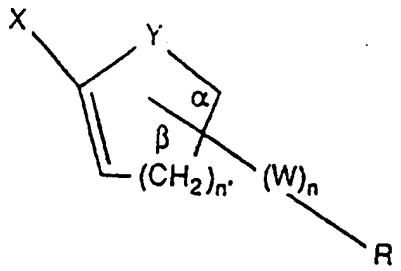
The buffe[redacted]olution of purified, derivatized 9.2.27 antibod[redacted]eoxygenated using a nitrogen sparge. The pH of the solution is then decreased to pH 5.0 using deoxygenated 0.1N HCl. The acidified antibody solution is kept at 5 ° C to minimize sulfhydryl oxidation and is used within 30 minutes.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

The features disclosed in the foregoing description, in the claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Claims

1. A method for producing a cleavable immunoconjugate, comprising the steps of:
reacting an agent, capable of addition to a carbon-carbon double bond, with a compound having the following general formula:



where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combinations thereof;
n is 0 to 10;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2; and
X is an H, alkyl group of C_6 or less, or alkoxy group of C_6 or less;
to form a derivatized agent; and
conjugating said derivatized agent with an antibody or fragment thereof to form said cleavable immunoconjugate.

2. The method of claim 1 wherein the agent is selected from the group consisting of drugs, toxins, biological response modifiers, radiodiagnostic compounds, radiotherapeutic compounds, and derivatives thereof.

3. The method of claim 2 wherein the toxin is selected from the group consisting of ricin, abrin, diptheria toxin, and Pseudomonas exotoxin A.

4. The method of claim 1 wherein R is an amino group, sulfhydryl group, hydroxyl group, carbonyl-containing group, or alkyl X′, where X′ is a leaving group.

5. The method of claim 1, additionally including, after the step of reacting, converting R of the derivatized agent to a group capable of reacting with an antibody or fragment thereof in preparation for conjugation with an antibody or fragment thereof.

6. The method of claim 1, additionally including, after the step of reacting, derivatizing an antibody or fragment thereof in preparation for conjugation with the derivatized agent.

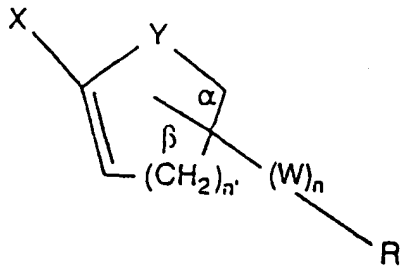
7. The method of claim 1 wherein the antibody or antibody fragment is monoclonal antibody or fragment thereof.

8. The method of claim 1 wherein the antibody fragments are $F(ab')_2$, Fab′, Fab, or Fv fragments.

9. The method of claim 1 wherein Y is an O, n′ is 1, and the antibody or antibody fragment is a monoclonal antibody or fragment thereof.

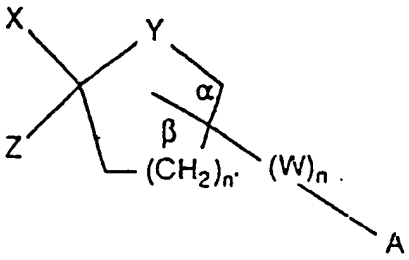
10. The method of claim 1 wherein the order of addition of an agent and an antibody or fragm nt thereof is reversed such that the antibody or fragment thereof is reacted with a compound of claim 1 via R and then the agent is conjugated via the carbon-carbon double bond of the compound.

11. A compound having the formula:



where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S, or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2; and
X is an H, alkyl group of C_6 or less, or alkoxy group of C_6 or less.

12. A cleavable immunoconjugate comprising an immunoconjugate having the following general formula:



where: A is an antibody including the linking function, or an antibody fragment including the linking function;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2;
X is an H, alkyl group of C_6 or less, or alkoxy group of C_6 or less; and
Z is an agent.

13. The cleavable immunoconjugate of claim 12 wherein the antibody or antibody fragment is a monoclonal antibody or fragment thereof.

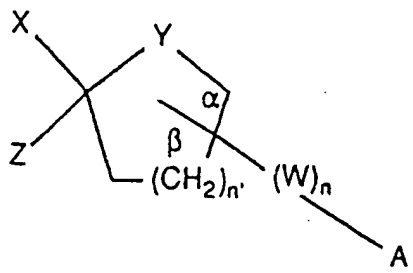
14. The cleavable immunoconjugate of claim 12 wherein the antibody fragments are $F(ab')_2$, Fab′, Fab, or Fv fragments.

15. The cleavable immunoconjugate of claim 12 wherein the agent is selected from the group consisting of drugs, toxins, biological response modifiers, radiodiagnostic compounds, radiotherapeutic compounds, and derivatives thereof.

16. The cleavable immunoconjugate of claim 15 wherein the toxin is selected from the group consisting of ricin, abrin, diptheria toxin, and Pseudomonas exotoxin A.

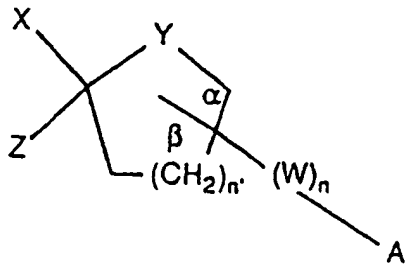
17. The cleavable immunoconjugate of claim 12 wherein Y is an O, n′ is 1, and the antibody or antibody fragment is a monoclonal antibody or fragment thereof.

18. A cleavable immunoconjugate having the following general formula:



where: A is an antibody including the linking function, or an antibody fragment including the linking function;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less; and
Z is an agent;
for use as an active therapeutic substance.

19. A cleavable immunoconjugate having the following general formula:



where: A is an antibody including the linking function, or an antibody fragment including the linking function;
W is a methylene, methylenoxy, or methylenecarbonyl group or combination thereof;
n is 0 to 10;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less; and
Z is a diagnostically or therapeutically effective agent;
for use within a method for delivering to the cytoplasm of a target cell an agent free of its antibody carrier.

20. The immunoconjugate of claim 19 wherein the antibody or antibody fragment is a monoclonal antibody or fragment thereof.

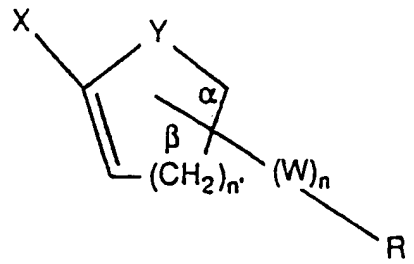
21. The immunoconjugate of claim 19 wherein the antibody fragments are $F(ab')_2$, Fab′, Fab, or Fv fragments.

22. The immunoconjugate of claim 19 wherein Y is an O, n′ is 1, and the antibody or antibody fragment is a monoclonal antibody or fragment thereof.

23. The immunoconjugate of claim 19 wherein the agent is selected from the group consisting of drugs, biological response modifiers, radiodiagnostic compounds, radiotherapeutic compounds, toxins, and derivatives thereof.

24. The immunoconjugate of claim 23 wherein the radiodiagnostic or radiotherapeutic compound contains a ^{99m}Tc, ^{186}Re, ^{188}Re, ^{131}I or ^{123}I radionuclide.

25. A method for isolating a compound, comprising the steps of:
conjugating to a solid phase a reagent having the following general formula:



where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combinations thereof;
n is 0 to 30;
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
n′ is 1 to 2; and
X is an H, alkyl group of C_6 or less, or alkoxy group of C_6 or less;
to form a derivatized solid phase; and
contacting said derivatized solid phase with a sample solution in which a compound containing an available nucleophilic group is present, such that said compound binds to said derivatized solid phase, thereby removing said compound from said sample solution.

26. The method of claim 25 wherein the solid phase is controlled pore glass, polyvinyl, polyacrylamide, polydextran, or polystyrene.

27. The method of claim 25 wherein R is an amino group, sulfhydryl group, or hydroxyl group, carbonyl-containing group, or alkyl X′, where X′ is a leaving group.

28. The method of claim 25 wherein Y is an O and n′ is 1.

29. The method of claim 25 wherein the available nucleophilic group of the compound is a sulfhydryl, amino, or hydroxyl group.

30. The method of claim 25, additionally including, after the step of contacting, washing the solid phase to remove noncovalently bound compounds.

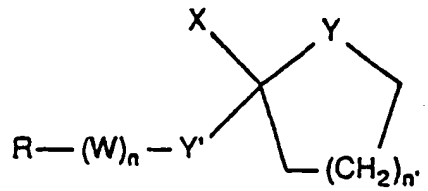
31. The method of claim 25, additionally including, after the step of contacting, releasing said bound compound from said derivatized solid phase.

32. The method of claim 31 wherein the step of releasing comprises releasing the bound compound by decreasing the pH to 6.0 or lower.

33. The method of claim 32 wherein the step of releasing further comprises releasing the bound compound by raising the temperature above 23° C.

34. The method of claim 31 wherein the step of releasing comprises releasing the bound compound by the addition of divalent cations.

35. A method for introducing into a compound a free sulfhydryl, free amino, or free hydroxyl group, comprising the steps of:
reacting a compound with a reagent having the following general formula



where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combinations thereof;
n is 0 to 30;
Y′ is S, NH, or O;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less;
n′ is 1 to 2; and
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less;
to form a reagent-linked compound; and

cleaving the [redacted]nt-linked compound at the bond between Y[redacted]the ring, thereby introducing into said compound said free sulfhydryl, free amino, or free hydroxyl group, depending upon whether Y′ is S, N, or O, respectively.

36. The method of claim 35 wherein R is an electrophilic group; Y is an O and n′ is 1.

37. The method of claim 35 wherein the compound is a protein.

38. The method of claim 37 wherein the protein is an antibody.

39. The method of claim 35 wher in th step of reacting comprises an amino group on the compound reacting with R of the reagent.

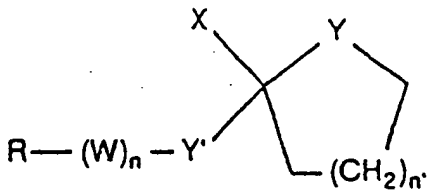
40. The method of claim 39 wherein Y′ is a S.

41. The method of claim 35 wherein the step of cleaving comprises exposing the reagent-linked compound to a pH of 6.0 or lower.

42. The method of claim 41 wherein the step of cleaving further comprises exposing the reagent-linked compound to a temperature above 23 ° C.

43. The method of claim 35 wherein the step of cleaving includes exposing the reagent-linked compound to divalent cations.

44. A compound having the formula:



where: R is a chemically reactive moiety;
W is a methylene, methylenoxy, or methylenecarbonyl group or combinations thereof;
n is 0 to 30;
Y′ is S, NH, or O;
X is H, alkyl group of C_6 or less, or alkoxy group of C_6 or less;
n′ is 1 to 2; and
Y is an O, S or NR′, wherein R′ is an alkyl group of C_6 or less.

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(54) **Cleavable immunoconjugates for the delivery and release of agents in native form.**

(57) The present invention provides a cleavable immunoconjugate whose linker contains a labile bond that is cleavable under a variety of mild conditions, including weakly acidic. Since the agent may be bonded directly to the linker, cleavage can result in release of native agent. The invention also provides methods for producing a cleavable immunoconjugate. Preferred agents include drugs, toxins, biological response modifiers, radiodiagnostic compounds, radiotherapeutic compounds, and derivatives thereof. The antibody employed in the invention may be an intact molecule, a fragment thereof, or a functional equivalent thereof. In a preferred embodiment, the specific antibody is a monoclonal antibody directed towards a tumor-associated antigen in man. The invention further provides a method for delivering to the cytoplasm of a target cell an agent free of its antibody carrier. A diagnostically or therapeutically effective dose of a cleavable immunoconjugate is administered to a mammal such as man.

Another aspect of the invention provides a method for isolating a compound containing an available nucleophilic group, such as a free sulfhydryl, amino, or hydroxyl group. The compound binds covalently to a solid phase which has been derivatized with the linker described above and is released in native form by a variety of mild conditions.

An additional aspect of the invention provides a method for introducing into a compound a free sulfhydryl, amine, or hydroxyl group by use of a reagent structurally related to the linker described above. Preferred uses of the method are to add a free amino or a free sulfhydryl group to a protein, such as an antibody. This method has broad application in the field of compound modification, especially protein modification.

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EUROPEAN SEARCH REPORT

Application number

EP 88 11 9966

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	WO-A-86 01 409 (DANA-FARBER CANCER INST. INC.) * Page 6, lines 1-35; claims * & US-A-4 569 789 (Cat. D) --	1-44	A 61 K 47/00 A 61 K 39/395 A 61 K 49/02
A	EP-A-0 115 171 (TEIJIN LTD) * Claims 6,7 * ----------	1-44	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	07-02-1989	BERTE

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